

Screening and Identification of Yeasts Antagonistic to Pathogenic Fungi Show a Narrow Optimal pH Range for Antagonistic Activity

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Abstract

Microbes have evolved ways of interference competition to gain advantage over their ecological competitors. The use of secreted antagonistic compounds by yeast cells is one of the prominent examples. Although this killer behavior has been thoroughly studied in laboratory yeast strains, our knowledge of the antagonistic specificity of killer effects in nature remains limited. In this study, yeast strains were collected from various niches and screened for antagonistic activity against one toxin-sensitive strain of *Saccharomyces cerevisiae* and three pathogenic fungi. We demonstrate that some strains with antagonistic activity against these pathogenic fungi can be found in antagonist culture tests. These yeasts were identified as members of *Trichosporon asahii*, *Candida stellimalicola*, *Wickerhamomyces anomalus*, *Ustilago esculenta*, *Aureobasidium pullulans*, and *Pichia kluyveri*. The results indicated that the antagonistic activity of these killer yeasts has a narrow optimal pH range. Furthermore, we found that the antagonistic activity of some species is strain-dependent.

Key words: antagonistic yeasts, interference competition, pathogenic fungi

Introduction

Two broad types of competition are generally recognized: interference and exploitative. Interference competition is a direct form of competition in which an organism actively interferes with another organism's ability to obtain resources. A common strategy for interference competition is the production of antibiotic compounds or toxins, by which cells inhibit the growth of sensitive competitors (Cornforth and Foster, 2013). The use of secreted antagonistic compounds by microbes is one prominent example (Burgess *et al.*, 1999; Jousset *et al.*, 2014; Schmitt and Breinig, 2002).

The most common method for selecting probiotic microbes involves testing the inhibition potential of the antagonistic compounds produced by candidate strains against known pathogens. Biological control using microbial agents has been suggested as an effective approach against pathogens, and antibiotics produced by microorganisms have garnered particular attention in research (Kohanski *et al.*, 2010; Mathur and Singh, 2005). Bacterial species producing antibiotics have been used as biocontrol agents against pathogenic fungi (Gebreel *et al.*, 2008; Yilmaz *et al.*, 2006). In addition, because antagonistic yeasts produce toxic secondary metabolites (antibiotics), they have been used in com-

mercial-scale yeast production including fermentation, formulation, storage, and handling (Wisniewski *et al.*, 2007; Wisniewski and Wilson, 1992).

Based on the aforementioned literature, this study investigated antagonistic activity against these human pathogenic fungi. We used single killer-toxin-sensitive strain of *Saccharomyces cerevisiae* (Chang *et al.*, 2015) and three pathogenic yeasts identified by the large subunit (LSU) of ribosomal DNA (rDNA) sequences isolated from the little black biting midge (*Forcipomyia taiwana*) (Chou *et al.*, 2015): *Aureobasidium pullulans* JYC1003, *Pseudozyma aphidis* JYC1050, and *Trichosporon asahii* JYC1040. To confirm these species, the ITS1-5.8S-ITS2 regions of their rDNA were also sequenced. These yeasts are emergent opportunistic pathogens that cause infections in immune-compromised individuals.

A. pullulans is classified as a dematiaceous fungus, commonly known as black yeast because of its melanin production. *A. pullulans* can cause corneal and dermal infections in immunocompromised patients (Girardi *et al.*, 1993). Despite the importance of *A. pullulans* in biotechnology (Molina-Leyva *et al.*, 2013), and although it rarely causes cutaneous infections in humans, several recent studies have reported its pathological significance (Molina-Leyva *et al.*, 2013; Vlchкова-Lashkoska *et al.*, 2004). *P. aphidis* is rarely

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reported as pathogenic; it is an opportunistic yeast typically isolated from plants and rarely from clinical specimens. Chen *et al.* (2011) isolated *P. aphidis* from the tissues of a patient with a swollen limb, and it has also been isolated from patients with astrocytoma (Hwang *et al.*, 2010). However, knowledge regarding the pathogenicity of *Pseudozyma* genus is limited. *Trichosporon* spp. are opportunistic pathogens that can cause invasive infections and are associated with a high mortality rate in immunocompromised patients (Girmenia *et al.*, 2005). *T. asahii* (previously *Trichosporon beigeli*) has wide infectious ability, even in healthy individuals; it can cause white piedra and hypersensitive pneumonia, as well as papular skin lesions, which are often fatal in immunocompromised patients (Bayramoglu *et al.*, 2008; Wolf *et al.*, 2001). To date, over 100 cases of hematogenously disseminated infections caused by this life-threatening yeast have been reported (Ebright *et al.*, 2001; Krcmery Jr *et al.*, 1999).

Because high levels of fungicide engender resistance in fungal pathogens, and because of growing public concerns over health and environmental hazards, new methods for controlling fungal diseases must be developed. Therefore, the objectives of this study were to screen and identify yeasts antagonistic to the aforementioned pathogenic fungi, and to investigate the influence of pH values on the antagonistic activity.

Experimental

Materials and Methods

Yeast isolation. The yeast samples were collected from various niches, including wine, flowers, insects and fruits and the like. They were transported to the laboratory in sealed plastic bags placed in an icebox and sent to the laboratory. The samples were maintained at a low temperature (4°C) prior to the yeast isolation procedures. The yeasts were isolated using an enrichment technique involving a malt extract medium (30 g/l of malt extract (Sigma-Aldrich, USA), 5 g/l of peptone (Sigma-Aldrich, USA)) supplemented with approximately 2–3 ml of 100% lactic acid (Sigma-Aldrich, USA). The samples were placed in 15-ml test tubes and incubated on a rotary shaker at 30°C for 3 h. A loopful of the enriched culture was streaked onto malt extract agar supplemented with approximately 2–3 ml of 100% lactic acid. Yeast colonies of different morphologies were selected and purified by cross-streaking on malt extract agar. Purified yeast strains were suspended in a YPD medium (1% yeast extract (Sigma-Aldrich, USA), 2% peptone, 2% dextrose (Sigma-Aldrich, USA)) supplemented with 15% v/v glycerol (Sigma-Aldrich, USA) and maintained at –80°C.

Yeast genomic DNA extraction. One milliliter of young yeast cultures was transferred to a 1.5-ml tube and centrifuged at 13,000–16,000 g for 1 min. The supernatant was discarded, and the cell pellet was suspended in 200 µl of a lysis buffer (2% Triton X-100 (Sigma-Aldrich, USA), 1% sodium dodecyl sulfate (Sigma-Aldrich, USA), 100 mM sodium chloride (Sigma-Aldrich, USA), 10 mM Tris (pH 8.0) (ACROS Organics, USA), and 1 mM ethylenediaminetetraacetic acid (EDTA) (Panreac Quimica, Spain)), to which 200 µl of phenol-chloroform-isoamyl alcohol (25:24:1, (Sigma-Aldrich, USA)) and 0.3 g of acid-washed glass beads (0.45–0.52 mm) was added and gently mixed. The samples were vortexed for 5 min to disrupt the cells and then centrifuged at 13,000–16,000 g for 5 min. The aqueous layer of each sample was then transferred to a clean tube, followed by the addition of 400 µl of 95% ethanol and 16 µl of 3M sodium acetate (pH 5.2) (Panreac Quimica, Spain). The samples were mixed through inversion and centrifuged at 13,000–16,000 g for 5 min. The pellets were then washed with 300 µl of 70% ethanol, and the samples were centrifuged at 13,000–16,000 g for 2 min before the supernatant was discarded. Subsequently, the ethanol solution was aspirated for 30 min to dry the pellets. Finally, genomic DNA from each sample was suspended in 100 µl of a Tris-EDTA buffer (pH 8.0).

Yeast identification. Sequences of the LSU rDNA were determined from the polymerase chain reaction (PCR) products of the genomic DNA extracted from the yeast cells. The LSU rDNA, including the D1/D2 domain, 5.8S rDNA, and internal transcribed spacer (ITS) regions, were amplified using a PCR with the universal primers ITS-1 (5'-TCCGTAGGTGAAC-CTGCG-3') and NL-4 (5'-GGTCCGTGTTTCAA-GACGG-3') (Kurtzman and Robnett, 1997). PCR proceeded as follows: initial denaturation at 95°C for 5 min followed by repeated denaturation at 95°C for 1 min, annealing at 48–55°C for 30 s, and elongation at 72°C for 1 min 40 s for 35 cycles; the final elongation lasted 5 min. The DNA sequencing of these samples was performed at Tri-I Biotech, Inc. A BLAST search of the nucleotide sequences was conducted through the National Center for Biotechnology Information homepage (<http://www.ncbi.nlm.nih.gov>). According to the guidelines of Kurtzman and Robnett (1998), yeast strains with 0–3 nucleotide differences among them were identified as conspecific or sister species, and those with >6 nucleotide substitutions as distinct species.

Analysis of the antagonistic activity. For the antagonistic activity assay, first, 100 µl of the lawn cell culture (approximately 1×10^7 cells) was spread on low-pH (pH 4.7, 5, 5.5, and 5.7) methylene blue (MB) plates (containing 1% yeast extract, 2% peptone, 0.1 M citric acid, and 0.003% MB dye). Two microliters of killer

cell culture (approximately 2×10^7 cells) was then spotted on the same plate. The lawn cells were one killer-toxin-sensitive strain of *S. cerevisiae* and three pathogenic fungi: *A. pullulans*, *T. asahii* and *P. aphidis*. MB is a dye that stains dead cells blue; living cells also absorb the dye, but the active enzymes within them process (reduce) the dye, rendering it colorless. This simple assay can be used for detecting yeast colonies containing dying or dead cells (Kucsera *et al.*, 2000). The MB plates in our study were incubated at 22°C for 4 d until a clear killing zone or colored dead colonies appeared.

Results

Yeast antagonists against *S. cerevisiae* and pathogenic fungi. In our study, 128 yeast strains isolated from samples of different natural sources were screened for antagonistic activity. The data showed that three isolates are antagonistic to the toxin-sensitive strain of *S. cerevisiae* (*T. asahii* JYC122 and JYC2122, and *Pichia kluyveri* JYC2095) (Fig. 1); three isolates are antagonistic to *A. pullulans* JYC1003 (*T. asahii* JYC122 and JYC2122, and *Wickerhamomyces anomalus* JYC2063) (Fig. 2); two isolates are antagonistic to *T. asahii* JYC1040 (*T. asahii* JYC122 and JYC2122) (Fig. 3); and five isolates are antagonistic to *P. aphidis* JYC1050 (*T. asahii* JYC122 and JYC2122, *Ustilago esculenta* JYC2036, *A. pullulans* JYC2095, and *Candida stellimalicola* JYC2120) (Figs. 4 and 5).

From the results, we determined that the killer *T. asahii* strains JYC122 and JYC2122 showed antagonistic activity against *T. asahii* JYC1040 at pH 4.7. These three isolates are different strains of the same species; hence, we also investigated whether *T. asahii* 1040 exhibits antagonistic activity against *T. asahii* JYC122 and JYC2122. Notably, *T. asahii* JYC1040 showed no

antagonistic activity under any of the pH conditions we tested (Fig. 6).

The influence of pH value on the antagonistic activity. In nature, yeasts live in fermenting organic material, which is generally acidic. Thus, most yeast

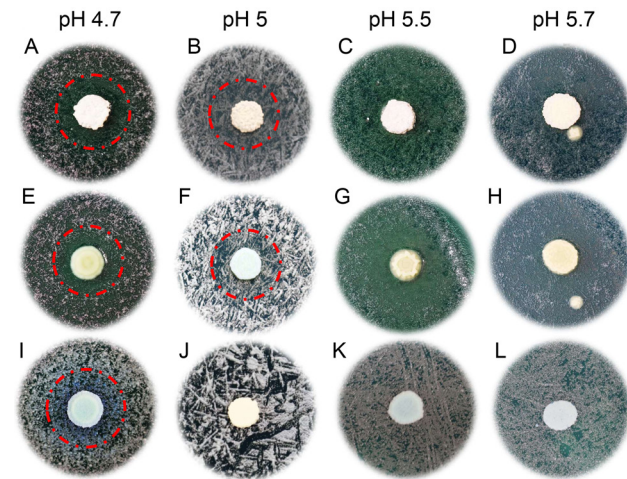


Fig. 2. Three isolates, *T. asahii* JYC122 (A-D) and JYC2122 (E-H), and *W. anomalus* JYC2063 (I-L), are antagonistic to *A. pullulans*.

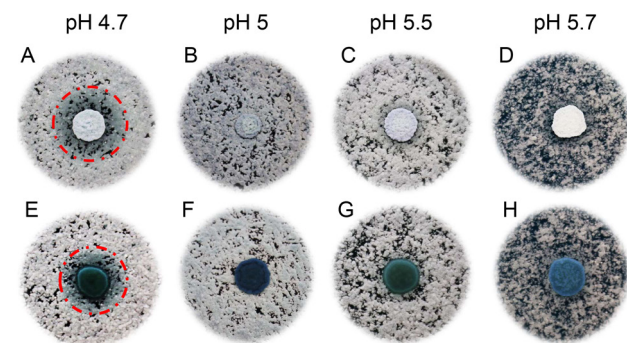


Fig. 3. Two isolates, *T. asahii* JYC122 (A-D) and JYC2122 (E-H), are antagonistic to *T. asahii* JYC1040.

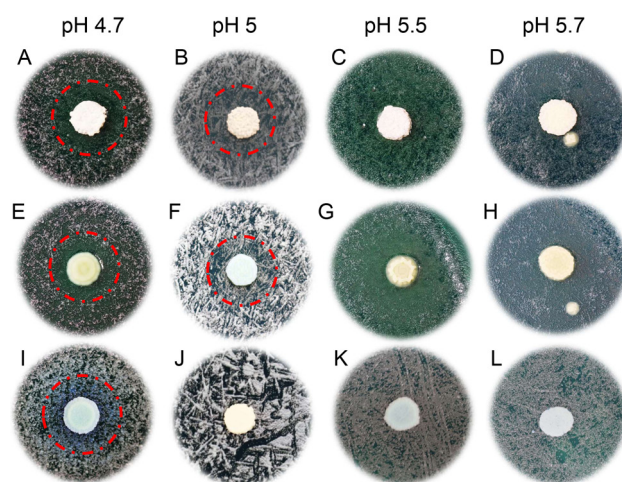


Fig. 1. *T. asahii* JYC122 (A-D) and JYC2122 (E-H), and *P. kluyveri* JYC2095 (I-L) are antagonistic to sensitive strains of *S. cerevisiae*.

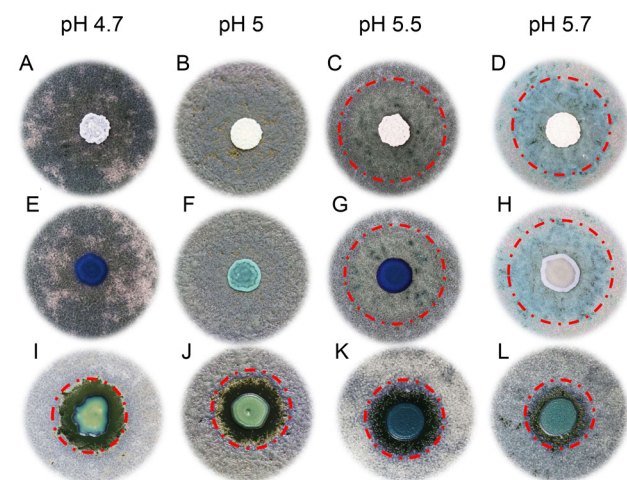


Fig. 4. Three isolates, *T. asahii* JYC122 (A-D) and JYC2122 (E-H), and *U. esculenta* JYC2036 (I-L), are antagonistic to *P. aphidis* JYC1050.

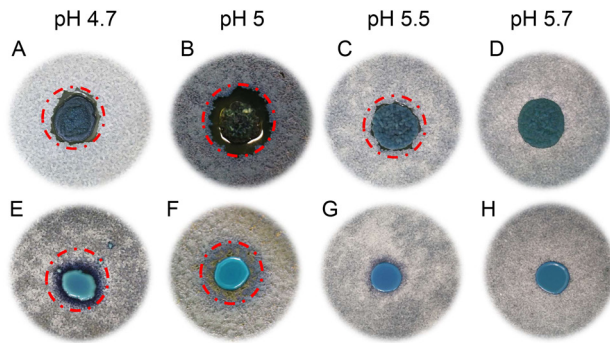


Fig. 5. Two isolates, *A. pullulans* JYC2095 (A-D) and *C. stellimalicola* JYC2120 (E-H), are antagonistic to *P. aphidis* JYC1050

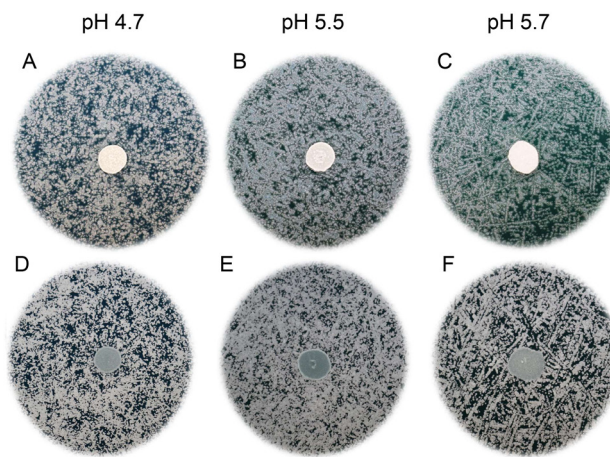


Fig. 6. *T. asahii* JYC1040 shows no antagonistic activity against *T. asahii* JYC122 (A-C) or JYC2122 (D-F) under any of the pH conditions tested.

media are mildly acidic. However, the fermentative activities of yeast alter the pH as the culture grows, which may change the antagonistic activity. Thus, we investigated the effect of pH on antagonism by observing the killing effects on MB plates incubated at 22°C for 4 d.

The inhibition haloes produced by *T. asahii* JYC122 and JYC2122 were clearer at pH 4.7 (Fig. 1A-E) and pH 5 (Fig. 1B-F) than at pH 5.5 (Fig. 1C-G) or pH 5.7 (Fig. 1D-H) when the lawn cells were a sensitive strain of *S. cerevisiae*. Furthermore, when the killer was *P. kluyveri* JYC2095, *S. cerevisiae* was surrounded by a blue precipitated halo – indicative of cellular death at pH 4.7 (Fig. 1I) – but not at pH 5, pH 5.5, or pH 5.7 (Fig. 1J-L). Similar results against *A. pullulans* JYC1003 were obtained from *T. asahii* JYC122 and JYC2122, and *W. anomalus* JYC2063 (Fig. 2). When the killer was *T. asahii* JYC122 or JYC2122, the lawn cells *T. asahii* JYC1040 were surrounded by a halo at pH 4.7 (Fig. 3A-E) but not at pH 5, pH 5.5, or pH 5.7 (Fig. 3B-D, F-H). Finally, when the lawn cells were *P. aphidis* JYC1050, *T. asahii* JYC122 and JYC2122 exerted stronger killer effects at pH 5.5 (Fig. 4C-G) and pH 5.7 (Fig. 4D-H) than at pH 4.7 (Fig. 4A-E) or pH 5

(Fig. 4B-F). *U. esculenta* JYC2036 exerted killer effect under all pH conditions we tested (Fig. 4I-L). *A. pullulans* JYC2095 exerted stronger killer effects at pH 4.7, pH 5, and pH 5.5 than at pH 5.7 (Fig. 5A-D). By contrast, *C. stellimalicola* JYC2120 showed the antagonistic behavior only at pH 4.7 and pH 5 (Fig. 5E-H).

These results indicated that the same lawn cells showed different sensitivities to different killer yeasts at the same pH value. Furthermore, the antagonistic activity of some killer yeasts had a narrow optimal pH range. However, several killer isolates that we found are also opportunistic pathogens (*T. asahii* JYC122 and JYC2122, and *A. pullulans* JYC2065). Despite the considerable biotechnological potential of these isolates, their pathogenicity should not be overlooked when considering them for future applications.

Strain-dependent antagonistic activity. We observed that *A. pullulans* shows antagonistic activity against *P. aphidis*. *A. pullulans* is a yeast-like fungus that can be found in different environments; it is notable for its phenotypic plasticity, and it is adaptable to various stressful conditions (e.g. hypersalinity, acidity, alkalinity, cold, and oligotrophy) (Slepecky and Starmer, 2009). Thus, antagonistic activity may be another characteristic of this polyextremotolerant species. Sixty-one *A. pullulans* strains were tested, and three of them (4.9%) were positive for the killer character (JYC1119, JYC1135, JYC1213). These strains were antagonistic to *P. aphidis* JYC1050 (Fig. 7), but not all the *A. pullulans* strains exhibited antagonistic activity against the other species we tested. Notably, *A. pullulans* JYC1135 and JYC1213 exerted stronger killer effects at pH 4.7, pH 5, and pH 5.5 than at pH 5.7 (Fig. 7E-L). However, *A. pullulans* JYC1119 exerted the killer effect only at pH 4.7 and pH 5, not at pH 5.5 or pH 5.7 (Fig. 7A-D). These results indicated the possibility that *A. pullulans* produces a unique antagonistic substance in response to *P. aphidis* but shows variety.

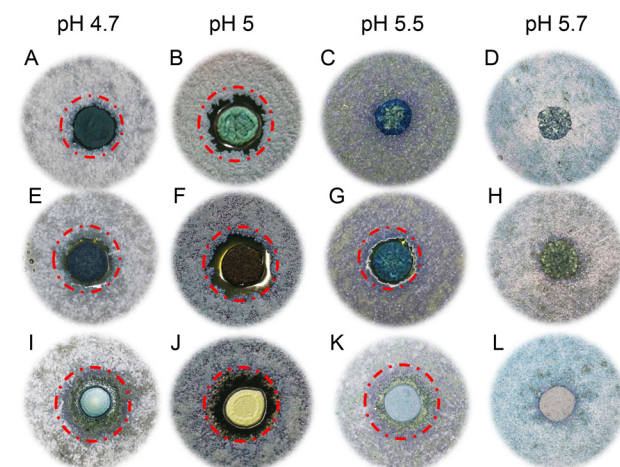


Fig. 7. *A. pullulans* JYC1119 (A-D), JYC1135 (E-H), and JYC1213 (I-L) exhibit antagonistic activity against *P. aphidis* JYC1050.

Discussion

The production of killer toxins is a well-established phenomenon in yeasts. The killer activity is detectable only when a suitable sensitive strain is tested and the killer strain is immune to its own toxin. The effect of a killer toxin depends on both its own potency and the susceptibility of lawn cells under selected conditions. Killer toxins are proteinaceous substances produced by some groups of yeast called “killer yeasts”, and these toxins kill sensitive strains of other yeasts. In general, they exhibit maximum activity under conditions of acidic pH (Liu *et al.*, 2013).

To evaluate the existence of a killer phenotype in a collection of yeast isolates, we used one toxin-sensitive strain of *S. cerevisiae* and three pathogenic fungi of potential clinical interest. The assays were performed within a narrow pH range likely to determine a killer phenotype, based on a previous study demonstrating that most killer toxins are stable and able to act only at acidic pH values. Kashiwagi *et al.* (1997) found that the SMK toxin secreted by the KK1 strain of the halo-tolerant yeast *Pichia farinosa* exhibits maximum killer activity under conditions of acidic pH and high salt concentration. The SMK toxin is composed of alpha and beta subunits, which tightly interact with each other under acidic conditions but are easily dissociated under neutral conditions and lose their killer activity. Similarly, the killer toxins of *Kluyveromyces wickerhamii* and *W. anomalus* maintain their killing activities in a pH range compatible with wine-making conditions and peak at pH 4.4 (Comitini *et al.*, 2004). However, some killer toxins are stable within a wide pH range. For example, the killer toxins produced by *Hansenula mrakii* are stable at pH 2–11, those by *Hansenula saturnus* at pH 3–11, and those by *Tilletiopsis albescens* at pH 3.5–8 (Marquina *et al.*, 2002).

In this study, *T. asahii* demonstrated broad killing activity against various yeast species. The broad killer phenomenon has been reported for other *Trichosporon* species including *Trichosporon japonicum* (Senter *et al.*, 2011) and *Trichosporon porosum* (Kulakovskaya *et al.*, 2010) and seems to be a common character in the genus (Golubev, 2006), although the killing phenomenon exhibited by *T. asahii* has yet to be investigated. The members of *Trichosporon* are fungi that commonly inhabit the soil, but several species occur as a natural part of the skin microbiota of humans and other animals (Chagas-Neto *et al.*, 2008; Zhang *et al.*, 2011). They belong to the basidiomycetous yeasts and have no sexual reproduction phase. Some yeast isolates exerted killer effects against more than one species, suggesting that the produced killer toxins could have a large spectrum of action. Alternatively, these yeasts could produce more than one killer toxin, similar to the

killer toxins K1, K2, and K28, produced by *S. cerevisiae* (Schmitt and Breinig, 2002).

In our study, several yeast strains were determined to be antagonistic to pathogenic fungi. Therefore, understanding how the toxins kill these pathogens is crucial, because the molecular mechanisms of this killer action could be helpful in developing strategies for fighting harmful fungi. In killer yeast, toxin secreting strains are frequently infected with double-stranded RNA viruses that are responsible for killer phenotype and toxin secretion in the infected host. Most viral toxins act as ionophores and disrupt cytoplasmic membrane function by forming cation-specific plasma membrane pores (such as K1, K2, and zygocin) that kill non-infected and sensitive yeast cells by disrupting cytoplasmic membrane function. In contrast, the *S. cerevisiae* K28 toxin enters susceptible cells by receptor-mediated endocytosis and act in the nucleus by blocking DNA synthesis and subsequently causing a G1/S cell cycle arrest (Liu *et al.*, 2013). Because the ability of yeasts to produce killer toxins is strain-dependent and the pH greatly influences toxin synthesis, it is also crucial for protein engineering must aim for toxin stability in a broader pH range.

In the future, these yeast antagonists can be used in treatments for fungal infections. The isolated antagonists can also be applied in food industries to reduce contamination by undesirable yeasts or other fungi.

Conclusion

The spectrum of action and the activity of yeast killer toxins are affected by temperature, salinity and pH of media. In the present work we determined the antagonistic activity against one toxin-sensitive strain of *S. cerevisiae* and three pathogenic fungi of yeasts isolated from various niches including wine, flowers, insects and fruits *et al.* In this study, we found that some strains with antagonistic activity against these pathogenic fungi can be found in antagonist culture tests. The assays were performed in a narrow range of pH, and this parameter shows a strong influence in the determination of killer phenotype. We further showed that the antagonistic activity of some species is strain-dependent.

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Conflict of Interests

The authors have not declared any conflict of interests.

Literature

- Bayramoglu G., M. Sonmez, I. Tosun, K. Aydin and F. Aydin. 2008. Breakthrough *Trichosporon asahii* fungemia in neutropenic patient with acute leukemia while receiving caspofungin. *Infection* 36(1): 68–70.
- Burgess J.G., E.M. Jordan, M. Bregu, A. Mearns-Spragg and K.G. Boyd. 1999. Microbial antagonism: a neglected avenue of natural products research. *J. Biotechnol.* 70(1): 27–32.
- Chagas-Neto T.C., G.M. Chaves and A.L. Colombo. 2008. Update on the genus *Trichosporon*. *Mycopathologia* 166(3): 121–132.
- Chang S.L., J.Y. Leu, and T.H. Chang. 2015. A population study of killer viruses reveals different evolutionary histories of two closely related *Saccharomyces sensu stricto* yeasts. *Mol. Ecol.* 24(16): 4312–22.
- Chen B., L.Y. Zhu, X. Xuan, L.J. Wu, T.L. Zhou, X.Q. Zhang and B.X. Li. 2011. Isolation of both *Pseudozyma aphidis* and *Nocardia otitidiscaviarum* from a mycetoma on the leg. *Int. J. Dermatol.* 50(6): 714–719.
- Chou J.Y., H.W. Chen, C.C. Lin, Y.D. Wen and W.L. Wang. 2015. Yeast diversity associated with the biting midge *Forcipomyia taiwana* in Taiwan. *Nova Hedwigia* 101(3–4): 519–527.
- Comitini F., N. Di Pietro, L. Zacchi, I. Mannazzu and M. Ciani. 2004. *Kluyveromyces phaffii* killer toxin active against wine spoilage yeasts: purification and characterization. *Microbiology* 150(Pt 8): 2535–2541.
- Cornforth D.M. and K.R. Foster. 2013. Competition sensing: the social side of bacterial stress responses. *Nat. Rev. Microbiol.* 11(4): 285–293.
- Ebright J.R., M.R. Fairfax and J.A. Vazquez. 2001. *Trichosporon asahii*, a non-Candida yeast that caused fatal septic shock in a patient without cancer or neutropenia. *Clin. Infect. Dis.* 33(5): e28–e30.
- Gebreel H., A. El-Mehalawy, I. El-Kholy, H. Rifaat and A. Humid. 2008. Antimicrobial activities of certain bacteria isolated from egyptian soil against pathogenic fungi. *Res. J. Agric. Biol. Sci.* 4(4): 331–339.
- Girardi L., R. Malowitz, G. Tortora, and E. Spitzer. 1993. *Aureobasidium pullulans* septicemia. *Clin. Infect. Dis.* 16(2): 338–339.
- Girmenia C., L. Pagano, B. Martino, D. D'Antonio, R. Fanci, G. Specchia, L. Melillo, M. Buelli, G. Pizzarelli and M. Venditti. 2005. Invasive infections caused by *Trichosporon* species and *Geotrichum capitatum* in patients with hematological malignancies: a retrospective multicenter study from Italy and review of the literature. *J. Clin. Microbiol.* 43(4): 1818–1828.
- Golubev W. 2006. Antagonistic interactions among yeasts, pp. 197–219. In: *Biodiversity and ecophysiology of yeasts*. Springer.
- Hwang S., J. Kim, S. Yoon, Y. Cha, M. Kim, D. Yong, J.H. Chang, S.H. Jeong, Y. Uh and K. Lee. 2010. First report of brain abscess associated with *Pseudozyma* species in a patient with astrocytoma. *Korean J. Lab. Med.* 30(3): 284–288.
- Jousset A., J. Becker, S. Chatterjee, P. Karlovsky, S. Scheu and N. Eisenhauer. 2014. Biodiversity and species identity shape the antifungal activity of bacterial communities. *Ecology* 95(5): 1184–1190.
- Kashiwagi T., N. Kunishima, C. Suzuki, F. Tsuchiya, S. Nikkuni, Y. Arata and K. Morikawa. 1997. The novel acidophilic structure of the killer toxin from halotolerant yeast demonstrates remarkable folding similarity with a fungal killer toxin. *Structure* 5(1): 81–94.
- Kohanski M.A., D.J. Dwyer and J.J. Collins. 2010. How antibiotics kill bacteria: from targets to networks. *Nat. Rev. Microbiol.* 8(6): 423–435.
- Krcmery Jr. V., F. Mateička, A. Kunová, S. Špánik, J. Gyarfás, Z. Syčová and J. Trupl. 1999. Hematogenous trichosporonosis in cancer patients: report of 12 cases including 5 during prophylaxis with itraconazol. *Support Care Cancer* 7(1): 39–43.
- Kucsera J., K. Yarita and K. Takeo. 2000. Simple detection method for distinguishing dead and living yeast colonies. *J. Microbiol. Methods* 41(1): 19–21.
- Kulakovskaya T.V., W.I. Golubev, M.A. Tomashevskaya, E.V. Kulakovskaya, A.S. Shashkov, A.A. Grachev, A.S. Chizhov and N.E. Nifantiev. 2010. Production of antifungal cellobiose lipids by *Trichosporon porosum*. *Mycopathologia* 169(2): 117–123.
- Kurtzman C.P. and C.J. Robnett. 1997. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large-subunit (26S) ribosomal DNA gene. *J. Clin. Microbiol.* 35(5): 1216–1223.
- Kurtzman C.P. and C.J. Robnett. 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* 73(4): 331–371.
- Liu G.L., Z. Chi, G.Y. Wang, Z.P. Wang, Y. Li and Z.M. Chi. 2013. Yeast killer toxins, molecular mechanisms of their action and their applications. *Crit. Rev. Biotechnol.* (0): 1–13.
- Marquina D., A. Santos, and J.M. Peinado. 2002. Biology of killer yeasts. *Int. Microbiol.* 5(2): 65–71.
- Mathur S. and R. Singh. 2005. Antibiotic resistance in food lactic acid bacteria – a review. *Int. J. Food Microbiol.* 105(3): 281–295.
- Molina-Leyva A., J.C. Ruiz-Carrascosa, A. Leyva-Garcia and H. Husein-Elahmed. 2013. Cutaneous *Cryptococcus laurentii* infection in an immunocompetent child. *Int. J. Infect. Dis.* 17(12): e1232–e1233.
- Schmitt M.J. and F. Breinig. 2002. The viral killer system in yeast: from molecular biology to application. *FEMS Microbiol. Rev.* 26(3): 257–276.
- Senter L., R.K.d.C. Ribas and P. Valente. 2011. Optimization of the cultivation conditions for the production of an antimicrobial compound by *Trichosporon japonicum* QU139. *Rev. Bras. Biocienc.* 9(1): 72–76.
- Slepecky R.A. and W.T. Starmer. 2009. Phenotypic plasticity in fungi: a review with observations on *Aureobasidium pullulans*. *Mycologia* 101(6): 823–832.
- Vlchikova-Lashkoska M., S. Kamberova, A. Starova, L. Goleva-Mishevskaa, N. Tsatsa-Biljanovska, V. Janevska and M. Petrovska. 2004. Cutaneous *Cryptococcus laurentii* infection in a human immunodeficiency virus-negative subject. *J. Eur. Acad. Dermatol. Venereol.* 18(1): 99–100.
- Wisniewski M., C. Wilson, S. Droby, E. Chalutz, A. El-Ghaouth, C. Stevens, C. Vincent, M. Goettel and G. Lazarovits. 2007. Post-harvest biocontrol: new concepts and applications, pp. 262–273. In: *Biological control: a global perspective*. CABI.
- Wisniewski M.E. and C.L. Wilson. 1992. Biological control of postharvest diseases of fruits and vegetables: recent advances. *Hort. Science* 27(2): 94–98.
- Wolf D.G., R. Falk, M. Hacham, B. Theelen, T. Boekhout, G. Scorzetti, M. Shapiro, C. Block, I.F. Salkin and I. Polacheck. 2001. Multidrug-resistant *Trichosporon asahii* infection of nongranulocytopenic patients in three intensive care units. *J. Clin. Microbiol.* 39(12): 4420–4425.
- Yilmaz M., H. Soran and Y. Beyatli. 2006. Antimicrobial activities of some *Bacillus* spp. strains isolated from the soil. *Microbiol. Res.* 161(2): 127–131.
- Zhang E., T. Sugita, R. Tsuboi, T. Yamazaki and K. Makimura. 2011. The opportunistic yeast pathogen *Trichosporon asahii* colonizes the skin of healthy individuals: analysis of 380 healthy individuals by age and gender using a nested polymerase chain reaction assay. *Microbiol. Immunol.* 55(7): 483–488.